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Hydropuntia edulis as a nutritional therapeutic agent for human melanoma: In silico approach and in vitro validation for functional food application

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ABSTRACT

Background: Melanoma is one of the deadliest forms of skin cancer, with rising incidence and limited responsiveness to conventional chemotherapy. Marine macroalgae have recently emerged as a promising source of novel bioactive compounds with potential anticancer properties.

Objective: This study aimed to investigate the anticarcinogenic and antioxidant potential of *Hydropuntia edulis* extract (HEE) using integrated metabolomic profiling, in silico predictions, and in vitro validation against melanoma.

Methods: Untargeted LC-HRMS metabolomic profiling was conducted to identify bioactive constituents in HEE. Key compounds were further analyzed through structure–activity relationship (SAR), ADMET, and molecular docking simulations targeting melanoma-related proteins (BRAF, AKT1, EGFR, and TYRO3). Antioxidant and antiproliferative effects were assessed using DPPH and MTT assays on B16-F10 melanoma cells.

Results: Several metabolites including Sangivamycin, Michosterol C, Linamarin, and Elaiomycin K were identified. SAR analysis showed high antineoplastic probability for Linamarin (Pa = 0.831), Sangivamycin (Pa = 0.730), and Michosterol C (Pa = 0.759). Michosterol C showed strong binding affinity to BRAF (−9.2 kcal/mol), comparable to Dabrafenib (−9.1 kcal/mol). HEE demonstrated dose-dependent DPPH radical scavenging and inhibited melanoma cell proliferation with IC50 values similar to positive controls.

Conclusion: This study highlights Hydropuntia edulis as a promising source of nutraceutical agents against melanoma. Through a combination of metabolomic profiling, computational prediction, and in vitro testing, HEE was shown to contain multiple bioactive compounds with antioxidant and antiproliferative potential. These findings suggest HEE could be further developed as a dietary supplement or functional food to support nutritional management of melanoma patients, rather than as a stand-alone chemotherapeutic. Future studies including compound isolation, in vivo validation, and clinical trials are needed to clarify its relevance, optimal dosing, and safety profile.

KEYWORDS

Hydropuntia edulis, melanoma, metabolomics, molecular docking, anticancer, antioxidant, marine bioactives

INTRODUCTION

Marine-derived bioresources are increasingly recognized for their role in pharmaceutical innovation, particularly in oncology[1]. Among them, macroalgae or seaweeds offer a vast chemical repertoire of secondary metabolites, including polyphenols, sulfated polysaccharides, terpenoids, and alka-

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loids, which contribute to their broad-spectrum biological activities[2]. In the Indonesian archipelago, Hydropuntia edulis (previously known as Gracilaria edulis), a red macroalga abundant in the eastern region, is traditionally utilized for food and commercial agar production, yet its pharmacological potential remains underexplored. However, evidence integrating seaweed bioactivity with clinical nutrition interventions for cancer patients remains scarce, representing a significant research gap that this study seeks to address[3].

The most aggressive type of skin cancer is melanoma. Over the past few decades, its incidence has been gradually increasing, making global healthcare systems face an increasing burden[4]. In 2020, there were an anticipated 325,000 new cases of melanoma worldwide (174,000 men and 151,000 women), and 57,000 deaths (32,000 men and 25,000 women). The burden of melanoma is predicted to rise to 510,000 new cases (an increase of almost 50%) and 96,000 fatalities (an increase of 68%), by 2040, if the 2020 rates hold true[5]. Melanoma represents a pressing global health concern due to its aggressive nature and resistance to standard chemotherapeutic regimens[6-8].

Mutations in oncogenic drivers such as BRAF, NRAS, and EGFR not only contribute to tumorigenesis but also offer molecular entry points for targeted therapy[9,10]. The application of anti- BRAF drugs can result in the suppression of cell growth[11]. Drugs that use immunotherapy have transformed the treatment of melanoma and greatly enhanced the prognosis of patients with advanced or metastatic cancer[12]. However, current inhibitors like Dabrafenib and Encorafenib often lead to adverse effects and resistance, necessitating the search for safer, multitarget natural alternatives[13]

Melanoma often shows a poor response to chemotherapy[14]. In this case, the investigation for new therapies is very important. Thus, *H. edulis* is highlighted in this area due to its numerous biological properties[15]. The present study evaluates the anticarcinogenic potential of H. edulis by employing an integrative approach that combines untargeted metabolomic profiling, SAR-based activity prediction, ADMET profiling, network pharmacology, molecular docking, and in vitro antioxidant and antiproliferative assays. This comprehensive strategy aims to unveil the multitarget mechanisms and nutritional therapeutic relevance of seaweed-derived compounds in melanoma treatment.

MATERIAL AND METHODS

Hydropuntia Edullis Preparation and Extraction

The plant sample used in this study was *H. Edullis* from Kupang, East Nusa Tenggara, Indonesia. Botanical identification and authentication of the samples, has been done using NCBI Taxonomy ID 172966 database (NCBI:txid172966). The study followed standard guidelines and regulations for plant-based in vitro experiments, Researchers also confirmed that the Indonesian local authorities already approved the sample collection. Additionally, the cell lines procedures also complied with the institutional ethical guidelines. First, The *H. Edullis* sample was thoroughly cleaned with fresh water to remove salt and sand, then freeze-dried. After drying, the sample was coarsely powdered using a mechanical grinder and stored in an airtight container at −20 °C until extraction. The homogenized G. edulis powder (100 g) was subjected to a three-time extraction process employing 70% methanol (1L) followed by a sonication method at 25 °C. Subsequently, the polysaccharides within the crude methanol extract were precipitated using 70% ethanol with a mass-to-volume ratio 1:25. After evaporating the ethanol, the resultant algal extract was then partitioned with hexane, chloroform, and ethyl acetate to increase polarity. Finally, the resulting four fractions, along with crude methanol extract, were dried under vacuum conditions using the BUCHI Rotavapor R-300[16].

Metabolites Profiling using Untargeted Metabolomic

LC/HRMS detects both major and minor constituents in natural extracts, providing a promising method for metabolomics and comprehensive metabolite discovery. The great sensitivity and efficiency of this method allow for the identification and validation of the chemical structures of plant metabolite molecules[17]. An untargeted metabolomic profiling of HEE, the analysis was conducted with employing Liquid Chromatography–High-Resolution Mass Spectrometry (LC-HRMS). For sample preparation, 50 µl of each extract was diluted with ethanol to a final volume of 1500 µl, followed by thorough mixing and filtration before injection into the LC-HRMS system. The instrumentation included a Thermo Scientific Dionex Ultimate 3000 RSLC nano HPLC equipped with dedicated columns and solvents, coupled with a Thermo Scientific Q Exactive mass spectrometer for high-resolution full scans and data-dependent MS/MS acquisition. This integrated approach enabled precise metabolite profiling of the seaweed extracts. The analytical protocols were adapted from previous work[18], with modifications to enhance metabolite solubility, improve chromatographic peak resolution, and obtain a more comprehensive metabolite profile, which was essential for this study's objective of elucidating H. edulis bioactive composition.

Specifically, we adjusted the extraction ratio (1:25 w/v instead of 1:20), applied sonication at 25 °C for 30 min rather than 20 min, and used an ethanol re-dissolution step before LC-HRMS analysis to enhance solubility and peak resolution[18].

In Silico Assessment

Prediction of phytochemical compound activities, and pharmacokinetic analysis

Assessment of the potential bioactivity of compounds identified from *H. Edullis* for antimelanoma and antioxidant treatment

used the WAY2DRUG PASS prediction tool (https://www.pharmaexpert.ru/passonline/predict.php), accessed on 05 April, 2024. This tool employs structure-activity relationship (SAR) analysis to compare the input compound with known compound in the database, to identify substances with specific potency[19]. A greater structural similarity between input compound and known compounds will lead to higher prediction score and comparable potential bioactivities. The Pa (Probability of Activity) value serves as the output prediction score, indicating the likelihood that the compound is active. A Pa value above 0.4 suggests that the com- pound has moderate potential. In our studies we use a cutoff score of 0.5 for evaluation purposes. The Pa value also reflects the precision, with higher Pa denoting increased accuracy[12]. Additionally, this study also evaluate the toxicity and drug like- ness of the compounds. ADMET (absorption, distribution, metabolism, excretion, and toxicity) parameters are used to evaluate how the compounds work across tissue and potential toxic effects[20]. Drug similarity was assessed according to Lipinski's Rule of Five (Ro5). Each ligand's properties were calculated using SMILES notation, with data sourced from ADMET Lab 2.0 (https://admetmesh.scbdd.com/service/evaluation/index) accessed on, and Protox II databases (https://tox-new.charite.de/protox_II/index.php?site=compound_input) accessed on April 05, 2024, alongside with the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) accessed on April 05, 2024[21].

Protein target identification and analysis

Target analysis of *H. Edullis* was performed using the SuperPred target analysis tool (https://prediction.charite.de/), accessed on April 05, 2024. Target prediction were obtained by entering SMILES notation on each compounds. For the SuperPred analysis, the cut-off scores were set at 80 % (on a scale of 0 to 100 %). Genes and protein associated with Melanoma were obtained from the Open Target database (https://opentargets.org/), accessed on April 05, 2024. A Venn diagram was then used to identify overlapping targets, the disease related targets (melanoma) and the *H. Edullis* extract. Using (https://bioinformatics.psb.ugent.be/webtools/Venn/), accessed on April 05, 2024.

Network pharmacology analysis

The goal of network pharmacology is to find genes linked to substances and illnesses, build a network of protein-protein interactions (PPIs), assess the network, and present the network 33. Version 12 of the STRING database (https://string-db.org/, accessed on April 14th, 2025) was utilized to investigate the interactions between the compound targets and the disease. This tool systematically compiles and integrates protein-protein interactions (PPIs) based on functional associations and physical interactions[22]. The analysis was conducted using "*Homo sapiens*" as the screening parameter, and the results were saved for further processing. All receptor substrates were included in the analysis, with a high confidence score threshold of 0.9 set to ensure robust interactions.

Molecular docking simulation

A computer method for predicting ligands' binding affinities to receptor proteins is called molecular docking. It can be used in nutraceutical research, but it has become a powerful tool for medication development[23]. Molecular docking simulation was carried out using Cavity-detection- guided Blind Docking (CB-Dock2). This method integrates homologous template fitting, docking, and cavity identification. CB-Dock 2 automatically identifies binding sites, determining the size and center, and adjusting the box's dimensions according to the ligands. CB-Dock2 also ranks the binding patterns according to Vina scores and provides 3D visualization of binding patterns. CurPocket (Curvature-based cavity detection technique) was carried out to further enhance the efficiency and accuracy of the docking process. The PubChem database was used to obtain the three-dimensional structure of target proteins[24]. RSV Protein Data Bank (https://www.rcsb.org) provided the receptor proteins, including BRAF(3OG7), AKT1 (4EKL), EGFR (1M17), and TYRO3 (1RHF). Before docking, CB2-Dock server automatically removed water molecules from the uploaded protein structures.

In Vitro Assessment

Antioxidant via DPPH Inhibition

Since oxidative stress is linked to a number of illnesses, such as cancer, heart disease, and neurological disorders, the antioxidant activity of HEE is of interest. Antioxidants can help shield cells from harm and lower the risk of certain illnesses by scavenging free radicals and preventing lipid peroxidation. All things considered, in-silico research indicates that HEE and its bioactive constituents possess strong antioxidant properties[25]. In order to quantify antioxidant capabilities, including the ability of compounds to act as hydrogen suppliers or free-radical scavengers (FRS), the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method is widely used, rapid, simple, and reasonably priced.

By eliminating DPPH, a stabilized free radical, the DPPH testing technique is linked to its removal[24]. Antioxidant activity was also assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the procedure by Nurkolis and Kurniawan[28,29]. All things considered, in-silico research indicates that HEE and its bioactive constituents possess strong antioxidant properties[26]. The DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. Sample concentrations of 45, 90, 135, 180, and 225µg/mL Seaweed Extract (HEE) were mixed with 3 mL of DPPH reagent in test vials and incubated at room temperature for 30 minutes. The reduction in DPPH concentration was determined by measuring absorbance at 517 nm. Trolox also was used as the positive control.

Evaluation of Antiproliferative Effects of HEE Using the MTT Assay

Cell toxicity research almost always uses the MTT test for measuring cellular metabolic activity[29]. The cytotoxic activity was evaluated using the MTT assay, following protocols established before[26], B16-F10 melanoma cells, harboring wild-type BRAF, were seeded into 96- well plates and incubated for 24 hours. The cells were then treated with green seaweed crude extract (HEE) at concentrations of 0, 100, and 300 µg/mL. Dabrafenib (Sigma-Aldrich, Darmstadt, Germany) served as the positive control and was administered under the same conditions, as described in previous studies. After treatment, both HEE and Dabrafenib were applied to the cells and incubated for another 24 hours. Post-incubation, the cells were washed with 1X PBS and subsequently treated with 100 µL of MTT solution (0.5 mg/mL). The plates were incubated at 37 °C for 30 minutes. Following this, 100 µL of DMEM stopper reagent was added to each well. Absorbance was measured at 550 nm using a microplate reader. To ensure reproducibility and minimize bias, all experiments were conducted in triplicate and repeated three times for each treatment group.

Data Management and Statistical Analyses

The data were analyzed using GraphPad Prism Premium 10 software (GraphPad Software, San Diego, CA, USA). IC_{50} values for antioxidant and cytotoxic assays were determined using non-linear regression (log(inhibitor) vs. normalized response, variable slope). Group differences between treatments and controls were evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. All experiments were performed in triplicate and expressed as mean ± SD.

RESULTS

Metabolites Identified in HEE via LC-HRMS

Untargeted metabolomic profiling of *Hydropuntia edulis* extract (HEE) using LC-HRMS successfully identified several bioactive compounds, including Sangivamycin, Sevadicin, Michosterol C, Linamarin, Elaiomycin K, and Tentoxin (Table 1). These compounds demonstrated diverse chemical structures and retention times, ranging from early elution for polar metabolites like Linamarin (RT ≈ 0.7–1.2 min) to late elution for more lipophilic compounds such as Michosterol C (RT ≈ 14.6 min). Notably, Sangivamycin, a known antimetabolite, appeared at a retention time of 0.798 min with a calculated mass of 309.1 g/mol and an (M+H)+ ion. The presence of multiple compounds with potential pharmacological activity suggests a complex and potentially synergistic chemical profile within the HEE.

In Silico Prediction of Anticancer Activity and Drug-Likeness

Structure–activity relationship (SAR) prediction via PASS Online revealed that Sangivamycin and Michosterol C possessed strong antineoplastic potential, with Pa scores of 0.730 and 0.759, respectively (Table 2). Sangivamycin also showed a high antimetabolite score (0.863), supporting its possible interference with DNA/RNA synthesis in cancer cells. Linamarin showed the highest Pa score (0.831), though its antimetabolite potential was lower (0.180). Toxicity profiles ranged from low (Class 6 for Linamarin, LD50 = 29,700 mg/kg) to moderate (Class 2 for Sangivamycin, LD50 = 11 mg/kg). Drug-likeness evaluation indicated that most compounds complied with Lipinski's, Pfizer, and GSK rules, although some (*e.g.*, Tentoxin) were rejected under GSK criteria, likely due to structural complexity or poor solubility.

Network Pharmacology or PPI Analysis

Figure 1 presents an integrated network pharmacology analysis. Figure 1A displays a Venn diagram illustrating overlapping molecular targets between HEE-derived compounds and melanoma- associated genes, signifying direct relevance to disease modulation. Figure 1B demonstrate KEGG pathway annotations, with enriched targets involved in multiple cancer-related pathways such as apoptosis, PI3K-Akt signaling, and MAPK signaling pathways.

Table 1. Metabolites observed HEE via Untargeted Metabolomic Profiling LC-HRMS

Name	Formula	Annot. DeltaMass (ppm)	Calc. MW	m/z	RT (min)	Reference Ion
Elaiomycin K	C11 H22 N2 O4	-1.68	246.1575	269.1467	8.438	(M+Na)+1
Michosterol C	C31 H54 O5	-2.13	506.3960	507.4033	14.637	(M+H)+1
Sangivamycin	C12 H15 N5 O5	-4.92	309.1058	310.1127	0.798	(M+H)+1
Sevadicin	C23 H26 N4 O4	-3.48	422.1939	421.1866	6.503	(M-H)-1
Tentoxin	C22 H30 N4 O4	4.56	414.2285	415.2358	12.858	(M+H)+1
Linamarin	**C10 H17 N O6**	**0.55**	**247.1057**	**246.0984**	**3.323**	**(M-H)-1**

Table 2. Evaluating metabolites in HEE potential for anticancer based on structure–activity relationship (SAR) predictions

Compounds	Pa Score*		Toxicity Model Computation Analysis**		Drug-Likeness***		
	Antineoplastic	Antineoplastic antimetabolite	Predicted LD50 (mg/kg)	Toxicity Class	Lipinski Rule	Pfizer Rule	GSK
Sangivamycin	0.730	0.863	11	2	Accepted	Accepted	**Accepted**
Sevadicin	-	-	80	3	Accepted	Accepted	**Rejected**
Michosterol C	0.759	-	3362	5	Accepted	Accepted	**Rejected**
Linamarin	0.831	0.180	29700	6	Accepted	Accepted	**Accepted**
Elaiomycin K	0.257	0.173	3140	5	Accepted	Accepted	**Accepted**
Tentoxin	**-**	**-**	**1000**	**4**	**Accepted**	**Accepted**	**Rejected**

*Way2Drug; **Protox; ***ADMET.

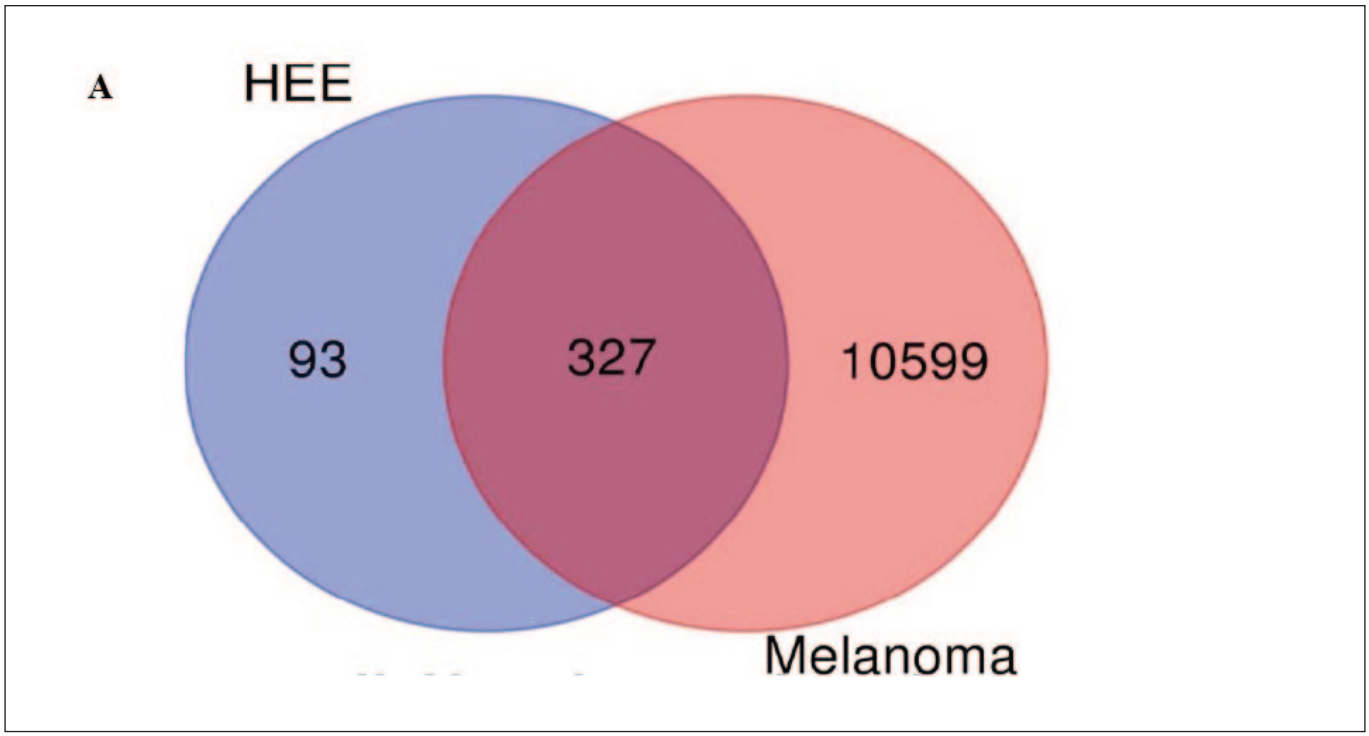

Figure 1A. Venn diagram of *Hydropuntia edulis extract* (HEE) and melanoma targets showing 327 overlapping genes as potential therapeutic targets

Integrative analysis using a bioinformatics approach was conducted to identify potential molecular targets of *Hydropuntia edulis* extract (HEE) in the context of melanoma cancer. From the initial mapping results, 420 target genes were predicted to interact with active compounds in HEE. After overlaying with gene expression data on melanoma, 327 overlapping genes were obtained, as visualized in the Venn diagram (Figure 1A). This shows that most of the potential targets of HEE play a role in biological pathways relevant to the pathophysiology of melanoma, strengthening the hypothesis that HEE may have a nutritional therapeutic effect on this type of cancer. Furthermore, an enrichment analysis was conducted on the 327 overlapping genes to evaluate their involvement in various signaling pathways based on the KEGG database.

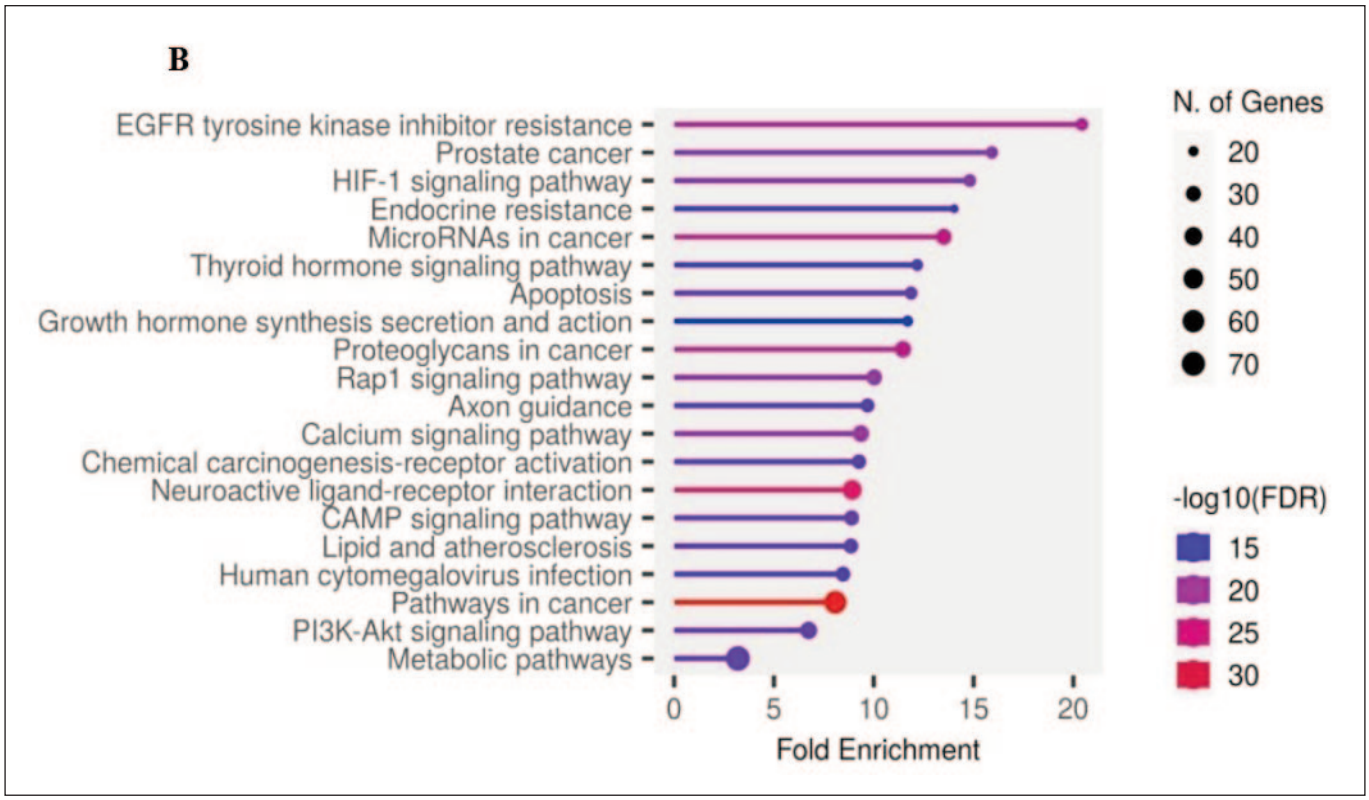

Figure 1B. Enriched KEGG pathways of overlapping targets between *Hydropuntia edulis extract* and melanoma, highlighting cancer-related signaling mechanisms

The analysis identified several pathways that were significantly ($p < 0.05$, FDR < 0.05) upregulated in relation to cancer regulation, including the EGFR tyrosine kinase inhibitors resistance pathway, HIF-1 signaling pathway, Endocrine resistance, MAPK signaling pathway, and other cancer pathways such as PI3K-Akt signaling pathway and Pathways in cancer (Figure 1B). The EGFR pathway ranked highest in fold enrichment, which is clinically relevant because mutations and dysfunction of this pathway have long been associated with therapy resistance and melanoma progression. The HIF-1 and PI3K-Akt pathways are also key components in the regulation of hypoxia, cell growth, and tumor cell survival, so the involvement of HEE target genes in these pathways provides a strong indication of potential multitarget anticancer activity.

Molecular Docking Scores of HEE Compounds Against Melanoma-Associated Proteins

Molecular docking simulations revealed favorable binding affinities between selected HEE compounds and key melanoma-related targets. Michosterol C demonstrated the strongest binding across multiple receptors, particularly BRAF (Vina score = -9.2) and EGFR (-7.9), suggesting potential for kinase inhibition. Sangivamycin also showed consistent moderate-to-strong affinity toward AKT1 (-7.7) and BRAF (-7.4). Compared to known melanoma drugs, Michosterol C's docking affinity to BRAF (-9.2) was comparable to Dabrafenib (-9.1), supporting its promise as a natural lead compound.

Antioxidant Activity

DPPH radical scavenging assays demonstrated a concentration-dependent antioxidant effect of HEE. The IC50 value calculated from the dose-response curve indicated moderate radical neutralization capacity, further supporting the extract's therapeutic relevance in oxidative stress- driven diseases such as melanoma.

An inhibition assay was conducted to evaluate the biological potential of the seaweed extract compared to Trolox, a standard antioxidant compound. The dose-response curves demonstrate that both Trolox and the seaweed extract exhibited increased inhibitory activity with rising logarithmic concentrations.

Quantitative analysis revealed that the IC_{50} value of Trolox was 84.88 µg/mL, whereas the seaweed extract had an IC_{50} of 116.1 µg/mL. This indicates that Trolox is more potent than the seaweed extract, requiring a lower concentration to inhibit 50% of the biological activity. However, the seaweed extract still showed biologically significant inhibi-

Table 3. The Vina score of the molecular docking parameter for the HEE compounds

Compounds and Control as Ligands	BRAF (3OG7)	AKT1 (4EKL)	EGFR (1M17)	TYRO3 (1RHF)	Overall
Control Daprafenib	-9.1	-7.6	-7.7	-7.1	-31.5
Control Encorafenib	-8.1	-7.3	-7	-6.7	-29.1
Control Dacarbazine	-6.1	-5.7	-5.4	-5.1	-22.3
Sangivamycin	-7.4	-7.7	-7.3	-6	-28.4
Michosterol C	-9.2	-7.4	-7.9	-7.1	-31.6

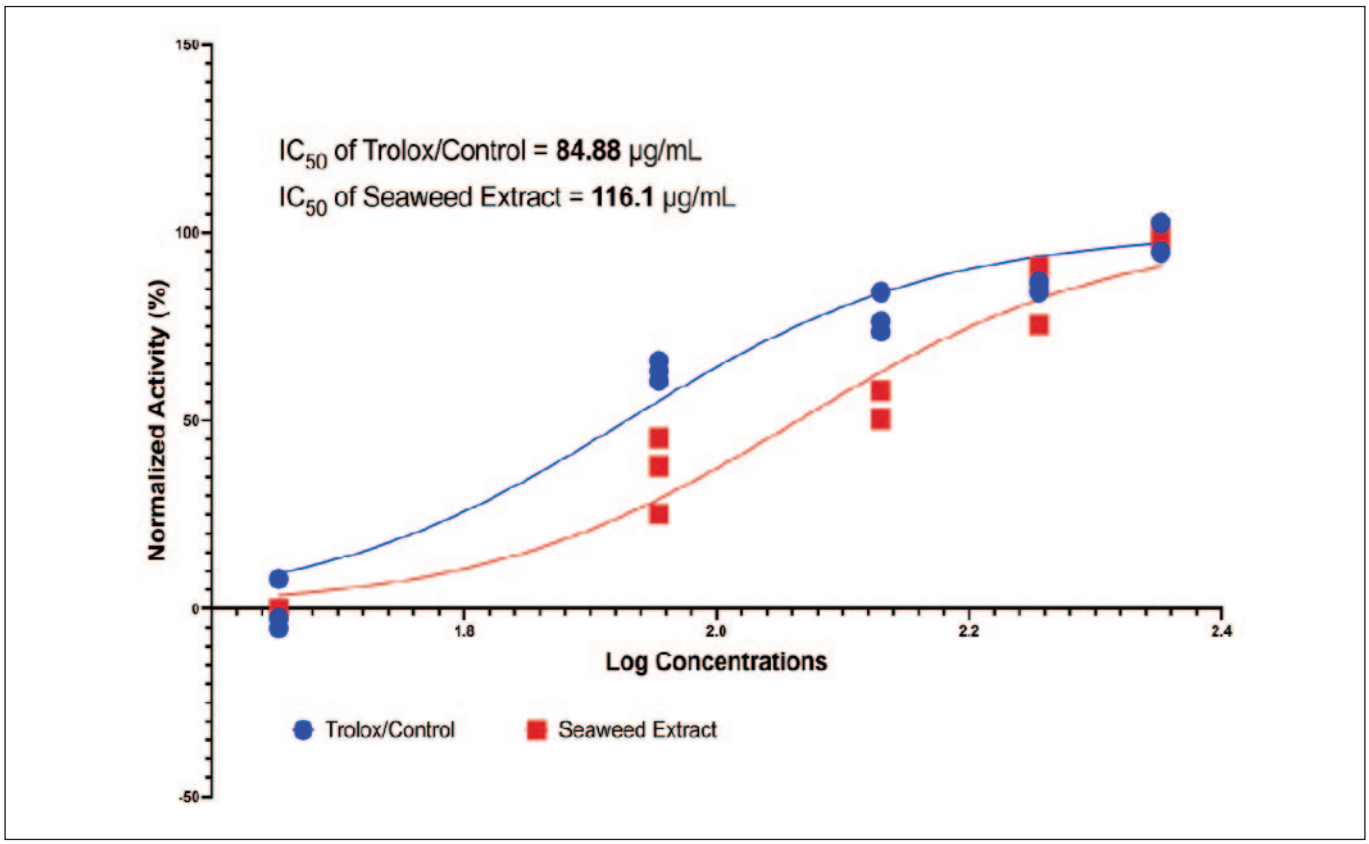

Figure 2. IC_{50} of DPPH Inhibition by HEE

tion, albeit at a higher concentration. The sigmoidal shape of the curve is characteristic of typical dose-response interactions, where increasing the concentration of the compound results in a higher percentage of inhibition. These findings suggest that although the seaweed extract is less potent than the reference standard, it still holds promise as a potential natural therapeutic agent particularly considering the complex, multitarget nature of marine-derived phytochemicals.

Antiproliferative Effect of HEE on B16-F10 Melanoma Cells

The MTT assay showed a dose-dependent cytotoxic effect of HEE on B16-F10 melanoma cells. At 217,2 µg/mL, HEE significantly reduced cell viability, approaching the inhibition levels seen with Dabrafenib, a clinically approved BRAF inhibitor. The IC50 value indicated that HEE exerts substantial antiproliferative activity, suggesting its potential as a complementary or alternative melanoma therapy.

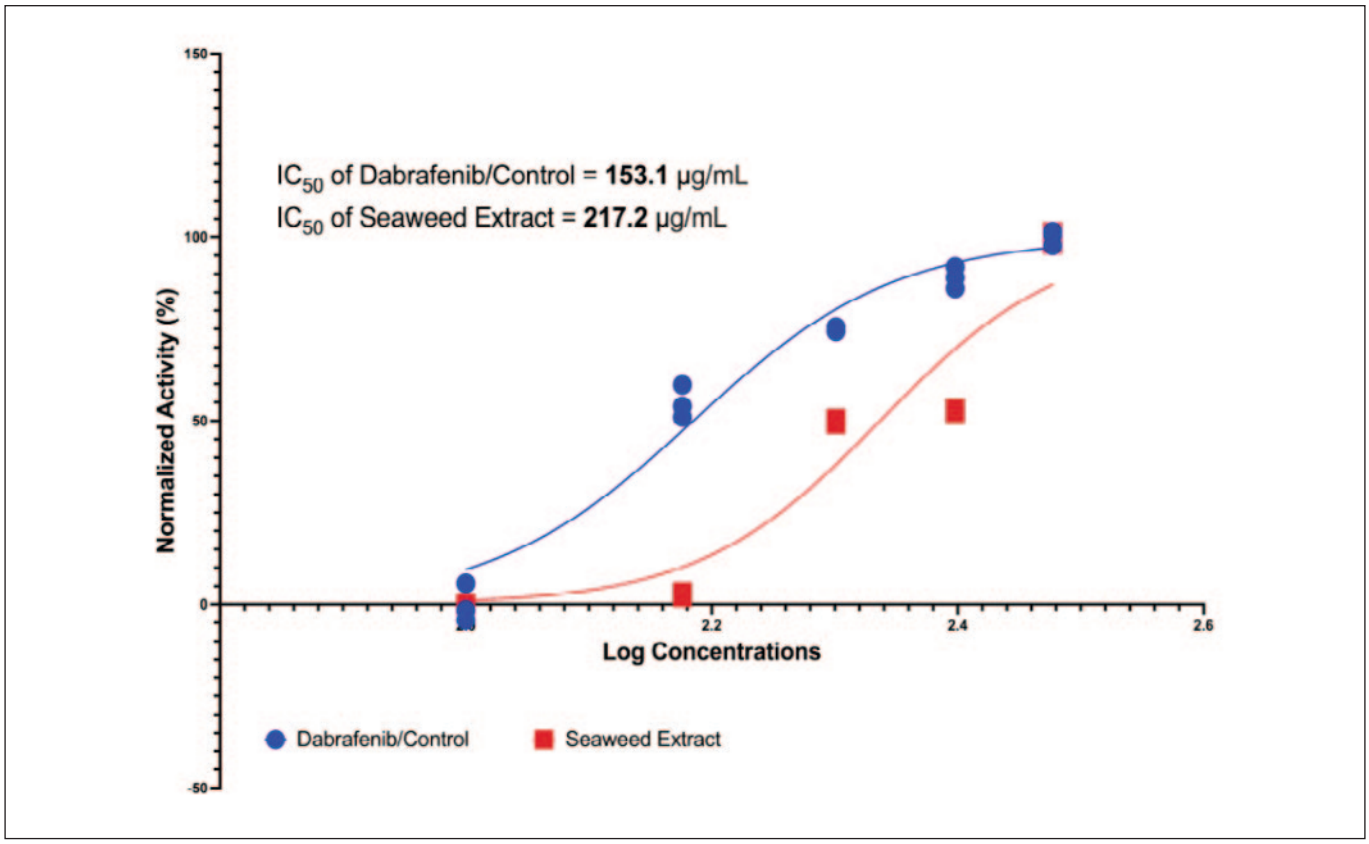

Figure 3. IC_{50} of B16-F10 melanoma cells Inhibition by HE

The dose-response analysis given in the graph compares the inhibitory effect of the seaweed extract to Dabrafenib, a well-known BRAF inhibitor used in melanoma treatment. Both drugs have a sigmoidal dose-response curve, indicating that the suppression of biological activity increases with concentration.

Dabrafenib had an IC_{50} value of 153.1 µg/mL, but the seaweed extract had a higher IC_{50} of 217.2 µg/mL. These findings imply that Dabrafenib is more effective at inhibiting the targeted biological activity than seaweed extract, as it takes a lower dosage to achieve 50% inhibition. Despite its decreased potency, the seaweed extract demonstrated significant biological activity.

DISCUSSION

The integration of metabolomic, computational, and cellular assays revealed compelling evidence for the nutritional therapeutic potential of *Hydropuntia edulis* extract (HEE) against melanoma. A highly important and hopeful method for addressing antioxidant and anticancer involves combating cellular pro-oxidant conditions by reducing the generation of reactive oxygen species (ROS) and the resulting oxidative stress[30]. Additionally, these seaweed bioactives exhibit immunomodulatory, antioxidant, and antiproliferative effects through distinct molecular mechanisms[31]. In Japan, Korea and China, seaweeds have served as important dietary components over past centuries. Historically, they have also been consumed in maritime communities across Europe and North America mainly in the diet of poor people living along the coastlines[32] However, evidence integrating seaweed bioactivity with clinical nutrition interventions for cancer patients remains scarce, representing a significant research gap that this study seeks to address.

Untargeted LC-HRMS profiling identified several pharmacologically relevant compounds, notably Sangivamycin, Michosterol C, and Linamarin. These molecules demonstrated promising antineoplastic properties as predicted by PASS online, with Pa scores exceeding 0.7, suggesting their mechanistic similarity to established anticancer agents. Positive Control Dabrafenib exhibited the strongest binding affinity with BRAF (-9.1) and TYRO3 (-7.1), achieving an overall affinity of -31.5. Encorafenib also demonstrated significant affinity towards all receptors, totaling -29.1. Dacarbazine presented the weakest affinity among the controls (-22.3), suggesting a lower potential for interaction in comparison to the other two controls.

Natural Test Compounds, michosterol C showed the strongest affinity for BRAF (-9.2) and boasted the highest total affinity value among all compounds at -31.6, surpassing Dabrafenib.

This highlights Michosterol C's impressive potential as an anti-melanoma agent. Sangivamycin exhibited a competitive binding characteristic with a total affinity of -28.4, approaching the control value of Encorafenib. Linamarin registered the lowest affinity among the natural compounds at -24.3, although it still outperformed Dacarbazine.

Michosterol C emerges as the most promising compound for therapeutic use concerning melanoma and oxidative stress, as it not only demonstrates a high affinity for crucial melanoma- related proteins (notably BRAF and EGFR) but also shows strong binding to the antioxidant receptor TYRO3. These results indicate that Michosterol C may function not just as an anticancer substance, but also as a facilitator of cellular oxidative balance.

Molecular docking simulations further validated the therapeutic hypothesis, with Michosterol C exhibiting a strong binding affinity to BRAF (−9.2 kcal/mol), outperforming even the clinical control Dabrafenib. Similarly, Sangivamycin showed robust interactions with AKT1 and EGFR. These results underscore the multitarget potential of HEE metabolites, which may provide an advantage over single-target inhibitors by simultaneously modulating several cancer-related pathways, including PI3K-Akt and MAPK signaling as supported by KEGG and STRING analyses[18].

The extract's in vitro efficacy was confirmed by its dose-dependent DPPH radical scavenging activity and significant cytotoxicity against B16-F10 melanoma cells, with inhibition comparable to Dabrafenib at the highest concentration. These findings align with previous reports on marine algal antioxidants, suggesting a dual mechanism involving both ROS scavenging and direct tumor cell inhibition[33].

Overall, these data support the hypothesis that HEE can act as a candidate anticancer nutritional therapeutic agent with the ability to intervene in various signaling pathways that are critical in the development and progression of melanoma. This makes HEE a promising material for further research, including in vitro and in vivo validation to confirm its pharmacological effects and toxicity. Despite its potential, this study has drawbacks, such as the absence of isolated compound testing and in vivo confirmation. However, the high convergence of cellular, metabolomic, and in silico data offers a solid basis for more research.

CONCLUSION

This study highlights *Hydropuntia edulis* as a promising source of anticancer agents against melanoma. Through a combination of untargeted metabolomic profiling, computational prediction, and in vitro testing, we demonstrated that HEE contains multiple bioactive compounds capable of targeting key melanoma-related pathways. Particularly, Michosterol C and Sangivamycin showed strong molecular docking affinity and significant cytotoxic effects. These findings warrant further pharmacological and preclinical evaluation to assess its full potential in melanoma therapy.

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